Active efflux mechanisms for antibiotic resistance: The tetracycline efflux protein

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Bacteria have intrinsic and acquired genes which specify traits which protect them from antibiotics. An increasingly more commonly-recognized mechanism is that of active efflux. There are a number of newly-discovered efflux systems for specific antibiotics such as chloramphenicol, the macrolides, and new ones for tetracyclines. There are other systems, such as the bmr-mediated membrane protein in Bacillus subtilis or norA in Staphylococcus aureus which appear to be involved in efflux of different antibiotics and toxic elements. The energy source for these active efflux systems comes from proton motive force (pmf) or from ATP.

The major focus of this laboratory has been the active efflux of tetracyclines mediated by the Tet(B) protein and other members of this family in the gram-negative bacteria. This twelve-membrane-spanning, inner membrane protein couples the exchange of a cation-tetracycline complex for a proton via energy provided from pmf, in particular ΔpH across the inner membrane. Tet protein serves as a model for other similar efflux proteins whose structure includes two halves (α and β) which have evolved as gene duplications. Mutations in either half eliminate resistance, but cells bearing separate polypeptides with mutations in either domain complement to give tetracycline resistance. Polypeptides consisting of only one domain of Tet protein will also act together to produce resistance. These data suggest that the two domains interact. A number of site-directed mutations have identified amino acids which affect resistance and efflux and may help define the substrate binding site.

Using semi-synthetic derivatives of tetracyclines, we have identified the minimum requirements for an analog which can competitively block Tet protein function. Such studies have generated in-depth analysis of active blocking agents with substitutions at C5 and C13 of the tetracycline molecule. A combination of a tetracycline with one of these blocking agents converts bacteria from a resistant to a susceptible phenotype. Of interest, the drug combination works against efflux systems in gram-positive and gram-negative bacteria and determinants of ribosomal protection. In view of the enlarging family of Tet-like proteins which efflux tetracyclines or other antibiotics, studies of Tet(B) protein will not only shed light on how to circumvent and block tetracycline efflux, but also define approaches to those homologous efflux systems which provide resistance to other drugs.